

AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph at page 5, lines 8-20, with the following:

Mammalian artificial chromosomes (MACs) are provided. Also provided are artificial chromosomes for other higher eukaryotic species, such as insects, birds, fowl and fish, produced using the MACS and methods provided herein. Methods for generating and isolating such chromosomes are provided. Methods using the MACs to construct artificial chromosomes from other species, such as insect, bird, fowl and fish species are also provided. The artificial chromosomes are fully functional stable chromosomes. Two types of artificial chromosomes are provided. One type, herein referred to as SATACs (satellite artificial chromosomes or satellite DNA based artificial chromosomes (the terms are used interchangeably herein)) are stable heterochromatic chromosomes, and the other type are minichromosomes based on amplification of euchromatin.

Please replace the paragraph beginning at page 11, line 11, through page 12, line 2, with the following:

These artificial chromosomes can be used in gene therapy, gene product production systems, production of humanized genetically transformed animal organs, production of transgenic plants and animals (non-human), including mammals, birds, fowl, fish, invertebrates, vertebrates, reptiles and insects, any organism or device that would employ chromosomal elements as information storage vehicles, and also for analysis and study of centromere function, for the production of artificial chromosome vectors that can be constructed in vitro, and for the preparation of species-specific artificial chromosomes. The artificial chromosomes can be introduced into cells using microinjection, cell fusion, microcell fusion, electroporation, nuclear transfer, electrofusion, projectile bombardment, nuclear transfer, calcium phosphate precipitation, lipid-mediated transfer systems and other such methods. Cells particularly suited for use with the artificial chromosomes include, but are not limited to plant cells, particularly tomato, arabidopsis, and others, insect cells, including silk worm cells, insect larvae, fish, reptiles, amphibians, arachnids, mammalian cells, avian cells, embryonic stem cells, ~~haematopoietic~~ hematopoietic stem cells, embryos and cells for use in methods of genetic therapy, such as lymphocytes that are used in methods

of adoptive immunotherapy and nerve or neural cells. Thus methods of producing gene products and transgenic (non-human) animals and plants are provided. Also provided are the resulting transgenic animals and plants.

Please replace the paragraph at page 17, lines 5-8, with the following:

As used herein, *in vitro* synthesized artificial chromosomes are artificial chromosomes that ~~[[is]]~~ are produced by joining the essential components (at least the centromere, and origins of replication) *in vitro*.

Please replace the paragraphs at page 19, lines 4-15, with the following:

As used herein, a SATAC refers to a chromosome that is substantially all heterochromatin, except for portions of heterologous DNA. Typically, SATACs are satellite DNA based artificial chromosomes, but the term ~~encompasses~~ encompasses any chromosome made by the methods herein that contains more heterochromatin than euchromatin.

As used herein, amplifiable, when used in reference to a chromosome, particularly the method of generating SATACs provided herein, refers to a region of a chromosome that is prone to amplification. ~~Amplification~~ Amplification typically occurs during replication and other cellular events involving recombination. Such regions are typically regions of the chromosome that include tandem repeats, such as satellite DNA, rDNA and other such sequences.

Please replace the paragraph beginning at page 22, line 22, through page 23, line 2, with the following:

As used herein, operative linkage of heterologous DNA to regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences refers to the relationship between such DNA and such sequences of nucleotides. For example, operative linkage of heterologous DNA to a promoter refers to the physical relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA in reading frame. Preferred promoters include tissue specific promoters, such as mammary gland specific promoters,

viral promoters, such as TK, CMV, adenovirus promoters, and other promoters known to those of skill in the art.

Please replace the paragraph at page 32, lines 13-24, with the following:

The resulting engineered minichromosome that contains the heterologous DNA can then be transferred by cell fusion into a recipient cell line, such as Chinese hamster ovary cells (CHO) and correct expression of the heterologous DNA can be verified. Following production of the cells, metaphase chromosomes are obtained, such as by addition of colchicine, and the chromosomes purified by addition of AT- and GC-specific dyes on a dual laser beam based cell sorter (see Example 10 B for a description of methods of isolating artificial ~~chromosomes~~ chromosomes). Preparative amounts of chromosomes (5×10^4 - 5×10^7 chromosomes/ml) at a purity of 95% or higher can be obtained. The resulting chromosomes are used for delivery to cells by methods such as microinjection and liposome-mediated transfer.

Please replace the paragraph at page 34, lines 8-27, with the following:

In particular, if a cell with a dicentric chromosome is selected, it can be grown under selective conditions, or, preferably, additional DNA encoding a second selectable marker is introduced, and the cells grown under conditions selective for the second marker. The resulting cells should include chromosomes that have structures similar to those depicted in Figures 2D, 2E, 2F. Cells with a structure, such as the sausage chromosome, Figure 2D, can be selected and fused with a second cell line to eliminate other chromosomes that are not of interest. If desired, cells with other chromosomes can be selected and treated as described herein. If a cell with a sausage chromosome is selected, it can be treated with an agent, such as BrdU, that destabilizes the chromosome so that the heterochromatic arm forms a chromosome that is substantially heterochromatic (*i.e.*, a megachromosome, see, Figure 2F). Structures such as the ~~gigachromosome~~ gigachromosome in which the heterochromatic arm has amplified but not broken off from the euchromatic arm, will also be observed. The megachromosome is a stable chromosome. Further manipulation, such as fusions and growth in selective conditions and/or BrdU treatment or other such treatment, can lead to

fragmentation of the megachromosome to form smaller chromosomes that have the amplicon as the basic repeating unit.

Please replace the paragraph beginning at page 38, line 22, through page 39, line 4, with the following:

(1) TF1004G-19C5

TF1004G-19C5 is a mouse LMTK- fibroblast cell line containing neo-minichromosomes and stable "sausage" chromosomes. It is a subclone of TF1004G19 and was generated by single-cell cloning of the TF1004G19 cell line. It has been deposited with the ECACC as an exemplary cell line and exemplary source of a sausage chromosome. Subsequent fusion of this cell line with CHO K20 cells and selection with hygromycin and G418 and HAT (hypoxanthine, ~~aminopterin~~ aminopterin, and thymidine medium; see Szybalski *et al.* (1962) *Proc. Natl. Acad. Sci.* 48:2026) resulted in hybrid cells (designated 19C5xHa4) that carry the sausage chromosome and the neo-minichromosome. BrdU treatment of the hybrid cells, followed by single cell cloning and selection with G418 and/or hygromycin produced various cells that carry chromosomes of interest, including GB43 and G3D5.

Please replace the paragraph beginning at page 52, line 22, through page 53, line 7, with the following:

Nuclear transfer (see, Wilmut *et al.* (1997) *Nature* 385:810-813, International PCT application Nos. WO 97/07669 and WO 97/07668). Briefly in this method, the SATAC containing the genes of interest is introduced by any suitable method, into an appropriate donor cell, such as a mammary gland cell, that contains totipotent nuclei. The diploid nucleus of the cell, which is either in G0 or G1 phase, is then introduced, such as by cell fusion or microinjection, into an unactivated oocyte, preferably enucleated cell, which is arrested in the metaphase of the second meiotic division. Enucleation may be effected by any suitable method, such as actual removal, or by treating with means, such as ultraviolet light, that functionally remove the nucleus. The oocyte is then activated, preferably after a period of contact, about 6-20 hours for cattle, of the new nucleus with the cytoplasm, while maintaining correct ploidy, to produce a reconstituted embryo, which is then introduced into

a host. Ploidy is maintained during activation, for example, by incubating the reconstituted cell in the presence of a microtubule inhibitor, such as nocodazole, colchicine, ~~ecemid~~ colcemid, and taxol, whereby the DNA replicates once.

Please replace the paragraph at page 57, lines 1-9, with the following:

The artificial chromosomes provided herein will be used in methods of protein and gene product production, particularly using insects as host cells for production of such products, and in cellular (*e.g.*, mammalian cell) production systems in which the artificial ~~chromosomes~~ chromosomes (particularly MACs) provide a reliable, stable and efficient means for optimizing the biomanufacturing of important compounds for medicine and industry. They are also intended for use in methods of gene therapy, and for production of transgenic plants and animals (discussed above, below and in the EXAMPLES).

Please replace the paragraph beginning at page 62, line 30, through page 63, line 11, with the following:

Thus, such cell-based "protein factories" employing MACs can be generated using MACs constructed with multiple copies (theoretically an unlimited number or at least up to a number such that the resulting MAC is about up to the size of a genomic chromosome (*i.e.*, endogenous) of protein-encoding genes with appropriate promoters, or multiple genes driven by a single promoter, *i.e.*, a fused gene complex (such as a complete metabolic pathway in plant expression system; see, *e.g.*, Beck von Bodman (1995) *Biotechnology* 13:587-591). Once such MAC is constructed, it can be transferred to a suitable cell culture system, such as a CHO cell line in protein-free culture medium (see, *e.g.*, (1995) *Biotechnology* 13:389-39) or other immortalized cell lines (see, *e.g.*, (1993) *TIBTECH* 11:232-238) where continuous production can be established.

Please replace the paragraph at page 78, lines 5-10, with the following:

These two cell lines, EC3/7C5 and EC3/7C6, thus carry a selectable mammalian minichromosome (MMCneo) with a centromere linked to a dominant marker gene (Hadlaczký *et al.* (1991) *Proc. Natl. Acad. Sci. U.S.A.* 88:8106-8110). MMCneo is intended to be used as a vector for minichromosome-mediated gene transfer and has been used as a model of a minichromosome-based vector system.

Please replace the paragraph beginning at page 83, line 23, through page 84, line 6, with the following:

The findings set forth in the above EXAMPLES demonstrate that the centromeric region of the mouse chromosome 7 has the capacity for large-scale amplification (other results indicate that this capacity is not unique to chromosome 7). This conclusion is further supported by results from cotransfection experiments, in which a second dominant selectable marker gene and a non-selected marker gene were introduced into EC3/7C5 cells carrying the formerly dicentric chromosome 7 and the neo-minichromosome. The EC3/7C5 cell line was transformed with λ phage DNA, a hygromycin-resistance gene construct (pH132), and a β -galactosidase gene construct (pCH110). Stable transformants were selected in the presence of high concentrations (400 μ g/ml) Hygromycin B, and analyzed by Southern hybridization. Established transformant cell lines showing multiple copies of integrated exogenous DNA were studied by in situ hybridization to localize the integration site(s), and by [[LacZ]] X-gal staining to detect β -galactosidase expression.

Please replace the paragraph at page 84, lines 9-24, with the following:

The pH132 plasmid carries the hygromycin B resistance gene and the anti-HIV-1 *gag* ribozyme (see, SEQ ID NO. 6 for DNA sequence that corresponds to the sequence of the ribozyme) under control of the β -actin promoter. This plasmid was constructed from pHyg plasmid (Sugden *et al.* (1985) *Mol. Cell. Biol.* 5:410-413; a gift from Dr. A. D. Riggs, Beckman Research Institute, Duarte; see, also, *e.g.*, U.S. Patent No. 4,997,764), and from pPC-RAG12 plasmid (see, Chang *et al.* (1990) *Clin Biotech* 2:23-31; provided by Dr. J. J. Rossi, Beckman Research Institute, Duarte; see, also U.S. Patent Nos. 5,272,262, 5,149,796 and 5,144,019, which describes the anti-HIV *gag* ribozyme and construction of a mammalian expression vector containing the ribozyme insert linked to the β -actin promoter and SV40 late gene transcriptional termination and polyA signals). Construction of pPC-RAG12 involved insertion of the ribozyme insert flanked by BamHI linkers [[was]] into BamHI-digested pH β -Apr-1gpt (see, Gunning *et al.* (1987) *Proc. Natl. Acad. Sci. U.S.A.* 84:4831-4835, see, also U.S. Patent No. 5,144,019).

Please replace the paragraphs beginnning at page 89, lines 6-22, with the following:

The expression of the non-selected β -galactosidase gene in the TF1004G-19C5 transformant was detected with [[LacZ]] X-gal staining of the cells. By this method one hundred percent of the cells stained dark blue, showing that there is a high level of β -galactosidase expression in all of TF1004G-19C5 cells.

2. The heterologous genes that are expressed are in the heterochromatin of the sausage chromosome

To demonstrate that the genes localized in the constitutive heterochromatin of the sausage chromosome provide the hygromycin resistance and the [[LacZ]] X-gal staining capability of TF1004G-19C5 transformants (*i.e.* β -gal expression), PEG-induced cell fusion between TF1004G-19C5 mouse cells and Chinese hamster ovary cells was performed. The hybrids were selected and maintained in HAT medium containing G418 (400 μ g/ml) and hygromycin (200 μ g/ml). Two hybrid clones designated 19C5xHa3 and 19C5xHa4, which have been deposited in the ECACC under Accession No. 96040927, were selected. Both carry the sausage chromosome and the minichromosome.

Please replace the paragraphs beginning at page 90, line 19, through page 91, line 16, with the following:

Since hygromycin-resistance and β -galactosidase genes were thought to be expressed from the sausage chromosome, the expression of these genes was analyzed in the four subclones that had lost the sausage chromosome. In the presence of 200 μ g/ml hygromycin, one hundred percent of the cells of four individual subclones died. In order to detect the β -galactosidase expression hybrid, subclones were analyzed by [[LacZ]] X-gal staining. One hundred percent of the cells of the four subclones that lost the sausage chromosome also lost the [[LacZ]] X-gal staining capability. All of the other hybrid subclones that had not lost the sausage chromosome under the non-selective culture conditions showed positive [[LacZ]] X-gal staining.

These findings demonstrate that the expression of hygromycin-resistance and β -galactosidase genes is linked to the presence of the sausage chromosome. Results of *in situ* hybridizations show that the heterologous DNA is expressed from the constitutive heterochromatin of the sausage chromosome.

In situ hybridization studies of three other hybrid subclones (G2C6, G2D1, and G4D5) did not detect the presence of the sausage chromosome. By the [[LacZ]] X-gal staining method, some stained cells were detected in these hybrid lines, and when these subclones were transferred to hygromycin selection some colonies survived. Cytological analysis and *in situ* hybridization of these hygromycin-resistant colonies revealed the presence of the sausage chromosome, suggesting that only the cells of G2C6, G2D1 and G4D5 hybrids that had not lost the sausage chromosome were able to preserve the hygromycin resistance and β -galactosidase expression. These results confirmed that the expression of these genes is linked to the presence of the sausage chromosome. The level of β -galactosidase expression was determined by the immunoblot technique using a monoclonal antibody.

Please replace the paragraph beginning at page 98, line 29, through page 99, line 4, with the following:

Liquid cultures of all 189 transformants were used to generate cosmid minipreps for analysis of restriction sites within the insert DNA. [[SIx]]Six of the original 189 cosmid clones contained an insert. These clones were designated as follows: 28 (~9-kb insert), 30 (~9-kb insert), 60 (~4-kb insert), 113 (~9-kb insert), 157 (~9-kb insert) and 161 (~9-kb insert). Restriction enzyme analysis indicated that three of the clones (113, 157 and 161) contained the same insert.

Please replace the paragraph at page 104, lines 12-22, with the following:

c. β -galactosidase β -galactosidase assay

The β -galactosidase assay mixture contained 1 mM $MgCl_2$, 45 mM β -mercaptoethanol, 0.8 mg/ml o-nitrophenyl- β -D-galactopyranoside and 66 mM sodium phosphate, pH 7.5. After incubating the reaction mixture with the cell extract at 37°C for increasing time, the reaction was terminated by the addition of three volumes of 1M Na_2CO_3 ,

and the optical density was measured at 420 nm. Assay mixture incubated without cell extract was used as a control. The linear range of the reaction was determined to be between 0.1-0.8 OD₄₂₀. One unit of β -galactosidase activity is defined as the amount of enzyme that will hydrolyse 3 nmoles of o-nitrophenyl- β -D-galactopyranoside in 1 minute at 37°C.

Please replace the paragraph at page 106, lines 6-8, with the following:

2. **Characterization of the β -galactosidase and hygromycin ~~phosphotransferase~~ phosphotransferase activity expressed in H1D3 and mM2C1 cells**

Please replace the paragraphs beginning at page 112, line 26, through page 113, line 23, with the following:

H1D3 has been fused with a CD4+ [[Hela]]HeLa cell line that carries DNA encoding CD4 and neomycin resistance on a plasmid (see, *e.g.*, U.S. Patent Nos. 5,413,914, 5,409,810, 5,266,600, 5,223,263, 5,215,914 and 5,144,019, which describe these [[Hela]]HeLa cells). Selection with GH has produced hybrids, including H1xHE41 (see Figure 4), which carries the megachromosome and also a single human chromosome that includes the CD4neo construct. Repeated BrdU treatment and single cell cloning has produced cell lines with the megachromosome (cell line 1B3, see Figure 4). About 25% of the 1B3 cells have a truncated megachromosome (~90-120 Mb). Another of these subclones, designated 2C5, was cultured on hygromycin-containing medium and megachromosome-free cell lines were obtained and grown in G418-containing medium. Recloning of these cells yielded cell lines such as IB4 and others that have a dwarf megachromosome (~150-200 Mb), and cell lines, such as I1C3 and mM2C1, which have a micro-megachromosome (~50-90 Mb). The micro-megachromosome of cell line mM2C1 has no telomeres; however, if desired, synthetic telomeres, such as those described and generated herein, may be added to the mM2C1 cell micro-megachromosomes. Cell lines containing smaller truncated megachromosomes, such as the mM2C1 cell line containing the micro-megachromosome, can be used to generate even smaller megachromosomes, *e.g.*, ~10-30 Mb in size. This may be accomplished, for example, by breakage and fragmentation of the micro-megachromosome in these cells

through exposing the cells to X-ray irradiation, BrdU or telomere-directed *in vivo* chromosome fragmentation.

EXAMPLE 8

Replication of the megachromosome

The homogeneous architecture of the ~~megachromomes~~ megachromosomes provides a unique opportunity to perform a detailed analysis of the replication of the constitutive heterochromatin.

Please replace the paragraph at page 130, lines 7-19, with the following:

Several procedures may be used to isolate metaphase chromosomes from these cells, including, but not limited to, one based on a polyamine buffer system (Cram *et al.* (1990) *Methods in Cell Biology* 33:377-382), one on a modified hexylene glycol buffer system (Hadlaczky *et al.* (1982) *Chromosoma* 86:643-65), one on a magnesium sulfate buffer system (Van den Engh *et al.* (1988) *Cytometry* 9:266-270 and Van den Engh *et al.* (1984) *Cytometry* 5:108), one on an acetic acid fixation buffer system (Stoehr *et al.* (1982) *Histochemistry* 74:57-61), and one on a technique utilizing hypotonic KCl and propidium iodide (Cram *et al.* (1994) XVII meeting of the International Society for Analytical Cytology, October 16-21, Tutorial IV *Chromosome Analysis and Sorting with ~~Commerieal~~ Commercial Flow Cytometers*; Cram *et al.* (1990) *Methods in Cell Biology* 33:376).

Please replace the paragraph at page 138, lines 13-24, with the following:

After transferring the constructs into the insect cell lines either by electroporation or by microinjection, expression of the marker genes was detected in luciferase assays (see *e.g.*, Example 12.C.3) and in β -galactosidase assays (such as [[lacZ]] X-gal staining assays) after a 24-h incubation. In each case a positive result was obtained in the samples containing the genes which was absent in samples in which the genes were omitted. In addition, a *B. mori* β -actin promoter-*Renilla* luciferase gene fusion was introduced into the *T. ni* and *B. mori* cells which yielded light emission after transfection. Thus, certain mammalian promoters function to direct expression of these marker genes in insect cells. Therefore, MACs are candidates for expression of heterologous genes in insect cells.

Please replace the paragraph beginning at page 145, line 25, through page 146, line 13, with the following:

In order to add PmeI and BglII sites to the synthetic telomere sequence, pTlk2 was digested with PacI and PstI and the 3.7-kb fragment (i.e., 2.7-kb pUC19 and 1.0-kb repeat sequence) was gel-purified and ligated at the PstI cohesive end with the following oligonucleotide 5'-GGGTTTAAACAGATCTCTGCA-3' (SEQ ID NO. 34). The ligation product was subsequently repaired with Klenow polymerase and dNTP, ligated to itself and transformed into *E. coli* strain DH5a. A total of 14 recombinants surviving selection on ampicillin were obtained. Plasmid DNA from each recombinant was able to be cleaved with BglII indicating that this added unique restriction site had been retained by each recombinant. Four of the 14 recombinants contained the complete 1-kb synthetic telomere insert, whereas the insert of the remaining 10 recombinants had undergone deletions of various lengths. The four plasmids in which the 1-kb synthetic telomere sequence remained intact were designated pTlkV2, pTlkV5, pTlkV8 and pTlkV12. Each of these plasmids could also be digested with PmeI; in addition the presence of both the BglII [[nad]] and PmeI sites was verified by sequence analysis. Any of these four plasmids can be digested with BamHI and BglII to release a fragment containing the 1-kb synthetic telomere sequence which is then ligated with BglII-digested pTEMPUD.

Please replace the paragraph at page 150, lines 14-27, with the following:

One means of achieving site-specific integration of heterologous genes into artificial chromosomes is through the use of homology targeting vectors. The heterologous gene of interest [[in]] is subcloned into a targeting vector which contains nucleic acid sequences that are homologous to nucleotides present in the artificial chromosome. The vector is then introduced into cells containing the artificial chromosome for specific site-directed integration into the artificial chromosome through a recombination event at sites of homology between the vector and the chromosome. The homology targeting vectors may also contain selectable markers for ease of identifying cells that have incorporated the vector into the artificial chromosome as well as lethal selection genes that are expressed only upon

extraneous integration of the vector into the recipient cell genome. Two exemplary homology targeting vectors, λ CF-7 and p λ CF-7-DTA, are described below.

Please replace the paragraph beginning at page 153, line 24, through page 154, line 5, with the following:

a. Construction of pJBP86

Plasmid pJBP86 was used in the construction of λ CF-7, above. A 1.5-kb Sall fragment of pCEPUR containing the puromycin-resistance gene linked to the SV40 promoter and polyadenylation signal was ligated to HindIII-digested pJB8 (see, *e.g.*, Ish-Horowitz *et al.* (1981) *Nucleic Acids Res.* 9:2989-2998; available from ATCC as Accession No. 37074; commercially available from Amersham, Arlington Heights, IL). Prior to ligation the Sall ends of the 1.5 kb fragment of pCEPUR and ~~the~~ HindIII linearized pJB8 ends were treated with Klenow polymerase. The resulting vector pJBP86 contains the puromycin resistance gene linked to the SV40 promoter and polyA signal, the 1.8 kb COS region of Charon 4A λ , the ColE1 origin of replication and the ampicillin resistance gene.

Please replace the paragraph at page 157, lines 3-12, with the following:

To obtain transfectant populations enriched in cells in which the luciferase gene had integrated into the minichromosome, transfected cells were grown in the presence of ~~ganciclovir~~ ganciclovir. This negative selection medium selects against cells in which the added pMCT-RUC plasmid integrated into the host EC3/7C5 genome. This selection thereby enriches the surviving transfectant population with cells containing pMCT-RUC in the minichromosome. The cells surviving this selection were evaluated in luciferase assays which revealed a more uniform level of luciferase expression. Additionally, the results of *in situ* hybridization assays indicated that the *Renilla* luciferase gene was contained in the minichromosome in these cells, which further indicates successful targeting of pMCT-RUC into the minichromosome.

Please replace the paragraph beginning at page 159, line 15, through page 160, line 2, with the following:

The initial source of the *R. reniformis* luciferase gene was plasmid pLXSN-RUC. Vector pLXSN (see, e.g., U.S. Patent Nos. 5,324,655, ~~5,470,730~~ 5,470,730, 5,468,634, 5,358,866 and Miller *et al.* (1989) *Biotechniques* 7:980) is a retroviral vector capable of expressing heterologous DNA under the transcriptional control of the retroviral LTR; it also contains the neomycin-resistance gene operatively linked for expression to the SV40 early region promoter. The *R. reniformis* luciferase gene was obtained from plasmid pTZrLuc-1 (see, e.g., U.S. Patent No. 5,292,658; see also the Genbank Sequence Database accession no. M63501; and see also Lorenz *et al.* (1991) *Proc. Natl. Acad. Sci. U.S.A.* 88:4438-4442) and is shown as SEQ ID NO. 10. The 0.97 kb EcoRI/SmaI fragment of pTZrLuc-1 contains the coding region of the *Renilla* luciferase-encoding DNA. Vector pLXSN was digested with and ligated with the luciferase gene contained on a pLXSN-RUC, which contains the luciferase gene located operably linked to the viral LTR and upstream of the SV40 promoter, which directs expression of the neomycin-resistance gene.

Please replace the paragraph at page 167, lines 4-12, with the following:

6) The capillary tip is lowered to the surface of the medium and is ~~foeussed~~ focused on the cells gradually until the tip of the capillary reaches the surface of a cell. The capillary is lowered further so that ~~[[the]]~~ it is inserted into the cell. Various parameters, such as the level of the capillary, the time and pressure, are determined for the particular equipment. For example, using the fibroblast cell line C5 and the above-noted equipment, the best conditions are: injection time 0.4 second, pressure 80 psi. DNA can then be automatically injected into the nuclei of the cells.

Please replace the paragraph beginning at page 172, line 27, through page 173, line 12, with the following:

Alternatively, the megachromosome-containing cell line G3D5* or H1D3* is fused with mouse embryonic stem cells (see, e.g., U.S. Patent No. 5,453,357, ~~commerically~~ commercially available; see *Manipulating the Mouse Embryo, A Laboratory Manual* (1994) Hogan *et al.*, eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pages 253-289) following standard procedures see also, e.g., *Guide to Techniques in Mouse Development in Methods in Enzymology* Vol. 25, Wassarman and De Pamphilis; eds. (1993),

pages 803-932). (It is also possible to deliver isolated megachromosomes into embryonic stem cells using the Microcell procedure (such as that described above).) The stem cells are cultured in the presence of a fibroblast (*e.g.*, STO fibroblasts that are resistant to hygromycin and puromycin). Cells of the resultant fusion cell line, which contains megachromosomes carrying the transgenes (*i.e.*, anti-HIV ribozyme, hygromycin-resistance and β -galactosidase genes), are then transplanted into mouse blastocysts, which are in turn implanted into a surrogate mother female mouse where development into a transgenic mouse will occur.

Please replace the paragraph at page 179, lines 9-24, with the following:

Alternatively, the artificial chromosomes may be introduced into chick zygotes, for example through direct microinjection (see, *e.g.*, Love *et al.* (1994) *Biotechnology* 12:60-63), which thereby are incorporated into at least a portion of the cells in the chicken. Inclusion of a tissue-specific promoter, such as an egg-specific promoter, will ensure appropriate expression of operatively-linked heterologous DNA.